EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2013. Scientific Opinion on Flavouring Group Evaluation 12, Revision 4 (FGE.12Rev4): primary saturated or unsaturated alicyclic alcohols, aldehydes, acids and esters from chemical groups 1 and 7

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SCIENTIFIC OPINION

Scientific Opinion on Flavouring Group Evaluation 12, Revision 4 (FGE.12Rev4): primary saturated or unsaturated alicyclic alcohols, aldehydes, acids and esters from chemical groups 1 and 7[1]

EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This scientific opinion, published on 5 December 2013, replaces the earlier version published on 16 October 2013*.

ABSTRACT

The Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids of the European Food Safety Authority was requested to evaluate 12 flavouring substances in Flavouring Group Evaluation 12, Revision 4 (FGE.12Rev4), including two additional substances, using the Procedure in Commission Regulation (EC) No 1565/2000. The present revision includes two additional flavouring substances: 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217]. None of the substances was considered to have genotoxic potential. The substances were evaluated through a stepwise approach (the Procedure) that integrates information on structure–activity relationships, intake from current uses and the toxicological threshold of concern and available data on metabolism and toxicity. The Panel concluded that none of the 12 substances [FL-nos: 02.134, 02.186, 02.216, 02.217, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829] gives rise to safety concerns at their levels of dietary intake, estimated on the basis of the maximised survey-derived daily intake approach. Besides the safety assessment of these flavouring substances, the specifications for the materials of commerce have also been considered. Specifications including complete purity criteria and identity for the materials of commerce have been provided for all 12 candidate substances.

KEY WORDS

primary alicyclic, saturated, unsaturated, alcohols, aldehydes, esters, flavourings

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[1] On request from the European Commission, Question No EFSA-Q-2013-00549, adopted on 25 September 2013.

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* Minor changes of editorial nature were made. The changes do not affect the contents of this report. To avoid confusion, the original version of the opinion has been removed from the website, but is available on request, as is a version showing all the changes made.

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SUMMARY

The European Food Safety Authority (EFSA) asked the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (the Panel) to deliver scientific advice to the Commission on the implications for human health of chemically defined flavouring substances used in or on foodstuffs in the Member States. In particular, the Panel was requested to evaluate 12 flavouring substances in the Flavouring Group Evaluation 12, Revision 4 (FGE.12Rev4), using the Procedure as referred to in Commission Regulation (EC) No 1565/2000. These 12 primary saturated or unsaturated alicyclic alcohol, aldehyde, acid and esters belong to chemical groups 1 and 7 of Annex I of Commission Regulation (EC) No 1565/2000.

The present revision of FGE.12, FGE.12Rev4, includes the assessment of two additional flavouring substances, 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217], compared with FGE.12Rev3.

Ten flavouring substances possess one or more chiral centres and additionally, owing to the presence of a double bond, three of these substances can exist as geometric isomers. For all 10 substances, the stereoisomeric composition has been specified sufficiently.

The 12 flavouring substances are classified into structural class I according to the decision tree approach presented by Cramer et al. (1978).

Four of the flavouring substances in the present group have been reported to occur in essential oils and in a few foods.

In its evaluation, the Panel, as a default, used the maximised survey-derived daily intake (MSDI) approach to estimate the per capita intakes of the flavouring substances in Europe. However, when the Panel examined the information provided by the European Flavouring Industry on the use levels in various foods, it appeared obvious that the MSDI approach would, in a number of cases, grossly underestimate the intake by regular consumers of products flavoured at the use level reported by the Industry, especially in those cases where the annual production values were reported to be small. In consequence, the Panel had reservations about the data on use and use levels provided and the intake estimates obtained by the MSDI approach.

In the absence of more precise information that would enable the Panel to make a more realistic estimate of the intakes of the flavouring substances, the Panel has decided also to perform an estimate of the daily intakes per person using a modified theoretical added maximum daily intake (mTAMDI) approach based on the normal use levels reported by Industry. In those cases where the mTAMDI approach indicated that the intake of a flavouring substance might exceed its threshold of concern, the Panel decided not to carry out a formal safety assessment using the Procedure. In these cases the Panel requires more precise data on use and use levels.

According to the default MSDI approach, intakes of the 12 flavouring substances in this group in Europe range from 0.011 to 43 micrograms (µg) per capita per day, i.e. below the threshold of concern value for structural class I (1 800 µg/person/day) substances.

The genotoxic potential of this group of flavouring substances cannot be fully assessed. However, the data available do not indicate a genotoxic potential and therefore do not preclude their evaluation via the Procedure.

The flavouring substances are expected to be metabolised to innocuous products at the estimated levels of intake as flavouring substances.

It is considered that on the basis of the default MSDI approach these 12 flavouring substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 2 to 5 000 μg/person/day for the 10 flavouring substances from structural class I for which use levels have been provided. For six of the substances the intakes were above the threshold of concern for structural class I of 1 800 μg/person/day. Thus, for six flavouring substances considered in this Opinion, the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for the structural class to which the flavouring substance has been assigned. For two substances [FL-no: 02.216 and 02.217], no use levels were provided. Therefore, for these eight substances [FL-nos: 02.134, 02.186, 02.216 and 02.217, 08.135, 09.342, 09.670 and 09.829] more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this Procedure, additional toxicological data might become necessary. The four substances whose intake estimates as determined by the mTAMDI are below the threshold of concern for structural class I are also expected to be metabolised to innocuous products.

In order to determine whether this evaluation could be applied to the material of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity tests for the materials of commerce have been provided for each of the 12 flavouring substances.

Thus, the Panel concluded that none of the 12 flavouring substances [FL-nos: 02.134, 02.186, 02.216, 02.217, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829] would present any safety concern at the estimated levels of intake based on the MSDI approach.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The use of flavourings is regulated under Regulation (EC) No 1334/2008 of the European Parliament and Council of 16 December 2008[4] on flavourings and certain food ingredients with flavouring properties for use in and on foods. On the basis of Article 9(a) of this Regulation, an evaluation and approval are required for flavouring substances.

The Union list of flavourings and source materials was established by Commission Implementing Regulation (EC) No 872/2012[5]. The list contains flavouring substances for which the scientific evaluation should be completed in accordance with Commission Regulation (EC) No 1565/2000[6].

EFSA has evaluated 11 flavouring substances, which correspond to subgroup 1.1.2 of FGE.19, in its evaluation of the flavouring group 201 (FGE.201). The opinion was adopted on 25 September 2008.

EFSA concluded that a genotoxic potential of the 11 α,β-unsaturated aldehydes and alcohol and related esters in the present FGE.201 could not be ruled out.

Information on one representative material 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] has now been submitted by the European Flavour Association. This information is intended to cover also the re-evaluation of the following four substances from FGE.19 subgroup 2.1 (FGE.207):

- 12-beta-Santalen-14-ol [FL-no: 02.216]
- 12-alpha-Santalen-14-ol [FL-no: 02.217]
- Santalyl acetate [FL-no: 09.034]
- Santalyl phenylacetate [FL-no: 09.712]

The Commission asks EFSA to evaluate this new information and depending on the outcome proceed to the full evaluation of the flavouring substances.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

The European Commission requests the European Food Safety Authority to carry out a safety assessment on the following five substances: 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217], santalyl acetate [FL-no: 09.034], santalyl phenylacetate [FL-no: 09.712] and 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], in accordance with Commission Regulation (EC) No 1565/2000.

[4] Regulation (EC) No 1334/2008 of the European Parliament and of the Council of 16 December 2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods and amending Council Regulation (EEC) No 1601/91, Regulations (EC) No 2232/96 and (EC) No 110/2008 and Directive 2000/13/EC. OJ L 354, 31.12.2008, p. 34-50.

[5] EC (European Commission), 2012. Commission implementing Regulation (EU) No 872/2012 of 1 October 2012 adopting the list of flavouring substances provided for by Regulation (EC) No 2232/96 of the European Parliament and of the Council, introducing it in Annex I to Regulation (EC) No 1334/2008 of the European Parliament and of the Council and repealing Commission Regulation (EC) No 1565/2000 and Commission Decision 1999/217/EC. OJ L 267, 2.10.2012, p. 1-161.

[6] Commission Regulation No 1565/2000 of 18 July 2000 laying down the measures necessary for the adoption of an evaluation programme in application of Regulation (EC) No 2232/96. OJ L 180, 19.7.2000, p. 8-16.

INTERPRETATION OF THE TERMS OF REFERENCE

The Flavour Industry had provided genotoxicity studies for the representative substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] and these data are considered by EFSA also to be representative for the substances [FL-no: 02.216 and 02.217]. Based on the new data, the Panel concluded in FGE.207 that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] does not give rise to concern with respect to genotoxicity. This conclusion can also be applied to the substances 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217], which will be evaluated through the Procedure in FGE.12Rev4.

ASSESSMENT

1. History of the evaluation of the substances in the present Flavouring Group Evaluation

The first version of the Flavouring Group Evaluation 12 (FGE.12) dealt with four primary saturated or unsaturated alicyclic alcohol, aldehyde, acid and esters.

The first revision of FGE.12, FGE.12Rev1, included the assessment of three additional candidate substances [FL-nos: 02.134, 05.137 and 05.198]. Additional information on two substances [FL-nos: 05.183 and 09.342] has been made available since FGE.12 was published.

The second revision of FGE.12, FGE.12Rev2, includes the assessment of two additional candidate substances [FL-nos: 08.135 and 09.829]. No toxicity and/or metabolism data were provided for these substances. Furthermore, for four substances [FL-nos: 02.186, 05.157, 05.198 and 09.670] information from Industry (EFFA, 2010) on stereoisomeric composition and missing specifications, received after publication of the last revision, was included in Revision 2.

The third revision of FGE.12, FGE.12Rev3, included the assessment of one additional candidate substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182]. No toxicity or metabolism data were provided for the substance. Furthermore, additional information on stereoisomeric composition for five substances [FL-nos: 02.186, 05.157, 05.182, 15.198 and 09.670], received after publication of Revision 2, was included in Revision 3.

FGE	Opinion adopted	Link	No of substances
FGE.12	23 February 2005	http://www.efsa.europa.eu/en/scdocs/scdoc/208.htm	4
FGE.12Rev1	28 August 2008	http://www.efsa.europa.eu/en/scdocs/scdoc/791.htm	7
FGE.12Rev2	30 September 2010	http://www.efsa.europa.eu/en/efsajournal/doc/1846.pdf	9
FGE.12Rev3	20 November 2012	http://www.efsa.europa.eu/en/efsajournal/pub/2993.htm	10
FGE.12Rev4	25 September 2013		12

The present Revision of FGE.12, FGE.12Rev4, includes the assessment of two additional flavouring substances, 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217]. These two substances have been considered with respect to genotoxicity in FGE.207 (EFSA CEF Panel, 2013) and the Panel concluded that the data available did rule out the concern for genotoxicity and thus concluded that the substances can be evaluated through the Procedure.

No toxicity or metabolism data were provided for the two substances. A search in the open literature did not provide any further relevant data on toxicity or metabolism for these substances.

2. Presentation of the substances in Flavouring Group Evaluation 12, Revision 4

2.1. Description

The present Flavouring Group Evaluation 12, Revision 4 (FGE.12Rev4), using the Procedure as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000) (the Procedure—shown in schematic form in Appendix A), deals with 12 candidate substances from chemical groups 1 and 7 of Annex I of Commission Regulation (EC) No 1565/2000 (EC, 2000).

The 12 flavouring substances under consideration, as well as their chemical Register names, FLAVIS (FL) numbers, Chemical Abstract Service (CAS) numbers, Council of Europe (CoE) numbers and Flavor and Extract Manufacturers Association (FEMA) numbers, structure and specifications, are given in Table 1.

Out of the 12 substances, one is a primary saturated alicyclic acid [FL-no: 08.135], three are esters, of which two [FL-nos: 09.342 and 09.670] have a primary saturated or unsaturated alicyclic alcohol moiety and one [FL-nos: 09.829] is an ethyl ester of a saturated alicyclic carboxylic acid, two substances [FL-nos: 02.134 and 02.186] are primary alicyclic saturated alcohols, two substances [FL-nos: 02.216 and 02.217] are primary alicyclic unsaturated alcohols and four are alicyclic unsaturated aldehydes [FL-nos: 05.157, 05.182, 05.183 and 05.198].

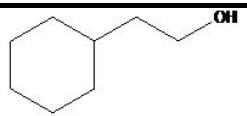
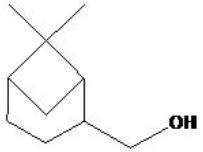
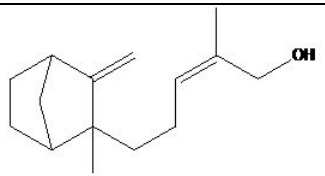
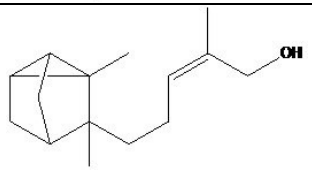
A summary of the safety evaluation is summarised in Table 5.

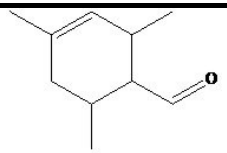
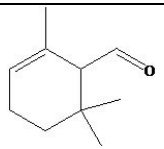
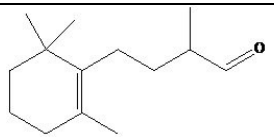
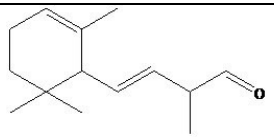
The 12 flavouring substances (candidate substances) are structurally related to 18 flavouring substances (supporting substances), 16 of which were evaluated at the 59th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (data available in Appendix B) as "Alicyclic Primary Alcohols, Aldehydes, Acids, and Related Esters", one of which was evaluated, also at the 59th JECFA meeting, as a member of the group "Phenethyl alcohol, aldehyde, acid and related acetals and esters" (JECFA, 2002a, 2003a) and one of which [FL-no: 09.931] was evaluated at the 61st JECFA meeting as being in the group "Aliphatic, branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters" (JECFA, 2004a, b). The supporting substances, along with their structural formulas, FEMA, CoE and CAS Registry numbers, status as evaluated by the Scientific Committee on Food (SCF), JECFA and CoE, and their European maximised survey-derived daily intake (MSDI) values, are listed in Table 7.

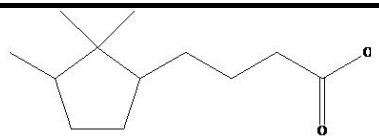
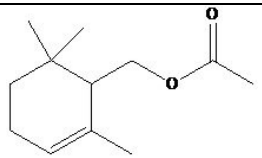
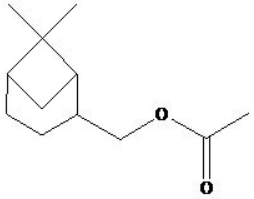
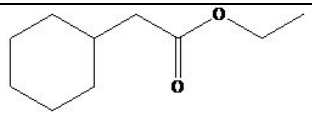
The hydrolysis products of the candidate esters are listed in Table 6.

SUMMARY OF SPECIFICATION DATA

Table 1: Specification summary of the substances in the Flavouring Group Evaluation 12, Revision 4

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [a] Solubility in ethanol [b]	Boiling point (°C) [c] Melting point (°C) Identification test Assay minimum	Refractive index [d] Specific gravity [e]	Specification comments
02.134	2-Cyclohexylethan-1-ol		 4442-79-9	Liquid $C_8H_{16}O$ 128.21	Slightly soluble Freely soluble	222 MS 95 %	1.463–1.469 0.918–0.924	
02.186	Myrtanol		 514-99-8	Solid $C_{10}H_{18}O$ 154.25	Practically insoluble or insoluble Freely soluble	116 (16 hPa) 77 MS 95 %	n.a. n.a.	Mixture of four diastereoisomers (EFFA, 2010). Four diastereoisomers, 20–30 % of each, with a higher likelihood of the trans forms (EFFA, 2012a)
02.216	12-beta-Santalen-14-ol		3006 74 77-42-9	Liquid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	129 (5.3 hPa) MS 95 %	1.498-1.509 0.965-0.975	EU Register name to be changed to beta-santalol and CAS Registry number to 81893-42-7. Mixture of diastereoisomers (EFFA, 2013a)
02.217	12-alpha-Santalen-14-ol		3006 74 115-71-9	Liquid $C_{15}H_{24}O$ 220.36	Insoluble Soluble	302 MS 95 %	1.498–1.509 0.965–0.975	Register name to be changed to alpha-santalol and CAS Registry number to 73307-63-8. Mixture of diastereoisomers (EFFA, 2013a)

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [a] Solubility in ethanol [b]	Boiling point (°C) [c] Melting point (°C) Identification test Assay minimum	Refractive index [d] Specific gravity [e]	Specification comments
05.157	Isocyclocitral		 1335-66-6	Liquid $C_{10}H_{16}O$ 152.23	Practically insoluble or insoluble Freely soluble	214 –55 MS 95 %	1.484–1.490 0.885–0.891	Mixture of two positional isomers (95 % sum of isomers, mainly 2,4,6-trimethyl-cyclohex-3-ene-1-carbaldehyde) (EFFA, 2010). Mixture of eight diastereoisomers (approximately 12.5 % each) (EFFA, 2012a)
05.182	2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde		3639 10326 432-24-6	Liquid $C_{10}H_{16}O$ 152.23	Insoluble Soluble	62 (0.4 hPa) MS 99 %	1.476–1.483 0.950–0.957	Racemate (EFFA, 2012a)
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal		 65405-84-7	Liquid $C_{14}H_{24}O$ 210.36	Practically insoluble or insoluble Freely soluble	305 MS 95 %	1.468–1.474 0.924–0.930	Racemate. CAS Registry number to be changed to 73398-85-3. New CAS Registry number refers to the racemate
05.198	alpha-Methyl ional		 58102-02-6	Liquid $C_{14}H_{22}O$ 206.33	Practically insoluble or insoluble Freely soluble	90 (0.1 hPa) MS 95 %	1.485–1.491 0.911–0.917	Mixture of (*Z*)- and (*E*)-isomers (EFFA, 2010). Name to be changed to 3-butenal, 2-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl) (EFFA, 2010). *E*-form (60–90 %); *Z*-form (10–40 %). In each case racemate (EFFA, 2012a)

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	Physical form Molecular formula Molecular weight	Solubility [a] Solubility in ethanol [b]	Boiling point (°C) [c] Melting point (°C) Identification test Assay minimum	Refractive index [d] Specific gravity [e]	Specification comments
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid		4529 957136-80-0	Liquid $C_{12}H_{22}O_2$ 198	Slightly soluble Partially soluble	140–143 NMR MS 99 %	1.461–1.467 0.955–0.961	Composition of mixture: 50–60 % 4-((1*S*,3*R*)-2,2,3-trimethylcyclopentyl)butanoic acid, 32–40 % 4-((1*R*,3*S*)-, 0–6 % 4-((1*S*,3*S*)-, 0–4 % 4-((1*R*,3*R*)-
09.342	Cyclogeranyl acetate		 54993-30-5	Liquid $C_{12}H_{20}O_2$ 196.29	Practically insoluble or insoluble Freely soluble	98 (13 hPa) MS 95 %	1.464–1.470 0.958–0.964	Racemate. CAS Registry number to be changed to 69842-11-1. New CAS Registry number refers to the racemate
09.670	Myrtanyl acetate		 29021-36-1	Liquid $C_{12}H_{20}O_2$ 196.29	Practically insoluble or insoluble Freely soluble	106 (9 hPa) MS 95 %	1.470–1.476 0.969–0.975	Mixture of *R*,*S*-enantiomers, i.e. the (+)- and (–)- (cis)- and (trans)-isomers, mixture of all diastereoisomers (EFFA, 2010). Four diastreoisomers 20–30 % each, with a higher likelihood of the trans forms (EFFA, 2012a)
09.829	Ethyl cyclohexyl acetate		2348 218 5452-75-5	Liquid $C_{10}H_{18}O_2$ 170.25	Practically insoluble or insoluble Freely soluble	211 NMR MS 95 %	1.442–1.450 0.945–0.948	

(a) Solubility in water, if not otherwise stated.
(b) Solubility in 95 % ethanol, if not otherwise stated.
(c) At 1013.25 hPa, if not otherwise stated.
(d) At 20 °C, if not otherwise stated.
(e) At 25 °C, if not otherwise stated.
n.a., not available; MS, mass spectrometry; NMR, nuclear magnetic resonance.

2.2. Stereoisomers

It is recognised that the geometrical and optical isomers of substances may have different properties. Their flavour may be different and they may have different chemical properties, resulting in possible variability in their absorption, distribution, metabolism, elimination and toxicity. Thus, information must be provided on the configuration of the flavouring substance, i.e. whether it is one of the geometrical/optical isomers or a defined mixture of stereoisomers. The available specifications of purity will be considered in order to determine whether the safety evaluation carried out for candidate substances for which stereoisomers may exist can be applied to the material of commerce. Flavouring substances with different configurations should have individual chemical names and codes (CAS number, FLAVIS number, etc.).

Ten of the 12 flavouring substances possess one or more chiral centres and additionally, owing to the presence of a double bond, three of these substances [FL-nos: 02.216, 02.217 and 05.198] can exist as geometric isomers. The stereoisomeric composition has been specified for all 10 substances (see Table 1).

2.3. Natural occurrence in food

Three of the 12 candidate substances, 2-cyclohexylethan-1-ol [FL-no: 02.134], myrtanol [FL-no: 02.186] and myrtanyl acetate [FL-no: 09.670], have been reported to occur in essential oils. One substance, 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], has been reported to occur naturally in grape brandy and tomato (TNO, 2012). No quantitative data were reported.

Eight of the substances (Table2) have not been reported to occur naturally in any food items according to TNO (2000, 2009, 2013).

Table 2: Candidate substances not reported to occur in food (TNO, 2000, 2009, 2013)

FL-no	Name
02.216	12-beta-Santalen-14-ol
02.217	12-alpha-Santalen-14-ol
05.157	Isocyclocitral
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal
05.198	alpha-Methyl ional
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid
09.342	Cyclogeranyl acetate
09.829	Ethyl cyclohexyl acetate

3. Specifications

Purity criteria for the 12 candidate substances have been provided by the Flavouring Industry (EFFA, 2003, 2004a, 2013a; Flavour Industry, 2009).

Judged against the requirements in Annex II of Commission Regulation (EC) No 1565/2000 (EC, 2000), the information is adequate for all 12 candidate substances (see Section 2.2 and Table 1).

4. Intake data

Annual production volumes of the flavouring substances as surveyed by the Industry can be used to calculate the MSDI by assuming that the production figure represents only 60 % of use in food owing to underreporting and that 10 % of the total EU population are consumers (SCF, 1999).

However, the Panel noted that, as a result of year-to-year variability in production volumes, uncertainties in the underreporting correction factor and uncertainties in the percentage of consumers, the reliability of intake estimates on the basis of the MSDI approach is difficult to assess.

The Panel also noted that, in contrast to the generally low per capita intake figures estimated on the basis of this MSDI approach, in some cases the regular consumption of flavoured products at use levels reported by the Flavour Industry in the submissions would result in much higher intakes. In such cases, the human exposure thresholds below which exposures are not considered to present a safety concern might be exceeded.

Considering that the MSDI model may underestimate the intake of flavouring substances by certain groups of consumers, the SCF recommended also taking into account the results of other intake assessments (SCF, 1999).

One of the alternatives is the TAMDI approach, which calculates intakes on the basis of standard portions and upper use levels (SCF, 1995) for flavourable beverages and foods in general, with exceptional levels for particular foods. This method is regarded as providing a conservative estimate of the actual intake of most consumers because it is based on the assumption that the consumer regularly eats and drinks several food products containing the same flavouring substance at the upper use level.

One option to modify the TAMDI approach is to base the calculation on normal rather than upper use levels of the flavouring substances. This modified approach is less conservative (e.g. it may underestimate the intake of consumers who are loyal to products flavoured at the maximum use levels reported) (EC, 2000). However, it is considered as a suitable tool to screen and prioritise the flavouring substances according to the need for refined intake data (EFSA, 2004b).

4.1. Estimated daily per capita intake (MSDI approach)

The intake estimation is based on the MSDI approach, which involves the acquisition of data on the amounts used in food as flavourings (SCF, 1999). These data are derived from surveys on annual production volumes in Europe. These surveys were conducted in 1995 by the International Organization of the Flavor Industry (IOFI). Flavour manufacturers reported the total amount of each flavouring substance incorporated into food sold in the EU during the previous year (IOFI, 1995). The intake approach does not consider the possible natural occurrence in food.

Average per capita intake (MSDI) is estimated on the assumption that the amount added to food is consumed by 10 % of the population[7] (Eurostat, 1998). This is derived for candidate substances from estimates of annual volume of production provided by Industry and incorporates a correction factor of 0.6 to allow for incomplete reporting (60 %) in the Industry surveys (SCF, 1999).

In the present Flavouring Group Evaluation (FGE.12Rev4), the total annual volume of production of the 12 candidate substances for use as flavouring substances in Europe has been reported to be approximately 370 kg (EFFA, 2003, 2004a, 2007, 2011; Flavour Industry, 2009). Of this amount, 350 kg is accounted for by one of these flavouring substances, 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135]. For the 18 supporting substances the total annual volume of production is approximately 380 kg (JECFA, 2003a; EFFA, 2013b).

On the basis of the annual volumes of production reported for the 12 candidate substances, the daily per capita intakes for each of these flavourings have been estimated (see Table 5).

[7] In the EU this amounts to 375 million. This figure relates to EU population at the time for which production data are available, and is consistent (comparable) with evaluations conducted prior to the enlargement of the EU. No production data are available for the enlarged EU.

The estimated daily per capita intake of 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135] from use as a flavouring substance is 43 µg. For each of the remaining substances the estimated daily per capita intake is less than 1 µg (see Table 5).

4.2. Intake estimated on the basis of the modified TAMDI (mTAMDI)

The method for calculation of mTAMDI values is based on the approach used by SCF up to 1995 (SCF, 1995).

The assumption is that a person may consume a certain amount of flavourable foods and beverages per day.

For 10 candidate substances, information on food categories and normal and maximum use levels[8,9,10] was submitted by the Flavour Industry (EFFA, 2003, 2004a, 2007, 2012b; Flavour Industry, 2009). No information on use levels has been submitted for 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217].

The 10 candidate substances are used in flavoured food products divided into the food categories, outlined in Annex III of Commission Regulation 1565/2000 (EC, 2000), as shown in Table 3. For the present calculation of mTAMDI, the reported normal use levels were used. If different use levels were reported for different food categories, the highest reported normal use level was used.

Table 3: Use of candidate substances in various food categories for 10 candidate substances for which data on use have been provided

Food category	Description	Flavourings used
01.0	Dairy products, excluding products of category 2	All except [FL-no: 05.182]
02.0	Fats and oils, and fat emulsions (type water-in-oil)	All except [FL-nos: 05.182, 08.135]
03.0	Edible ices, including sherbet and sorbet	All except [FL-no: 08.135]
04.1	Processed fruits	All except [FL-no: 08.135]
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	None
05.0	Confectionery	All
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery wares	All except [FL-nos: 05.182, 08.135]
07.0	Bakery wares	All except [FL-no: 08.135]
08.0	Meat and meat products, including poultry and game	All except [FL-nos: 05.182, 08.135]
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	All except [FL-nos: 05.182, 08.135]
10.0	Eggs and egg products	None
11.0	Sweeteners, including honey	Only [FL-no: 08.135]
12.0	Salts, spices, soups, sauces, salads, protein products, etc.	All except [FL-nos: 05.182, 08.135]

[8] "Normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002).
[9] The normal and maximum use levels in different food categories (EC, 2000) have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004b).
[10] The use levels from food category 5, "Confectionery", have been inserted as default values for food category 14.2, "Alcoholic beverages" ,for substances for which no data have been given for food category 14.2 (EFFA, 2007).

Food category	Description	Flavourings used
13.0	Foodstuffs intended for particular nutritional uses	All except [FL-nos: 05.182, 08.135]
14.1	Non-alcoholic ("soft") beverages, excluding dairy products	All
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	All except [FL-no: 05.182]
15.0	Ready-to-eat savouries	All except [FL-nos: 05.182, 08.135]
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 1–15	All except [FL-no: 05.182]

According to the Flavour Industry, the normal use levels for the candidate substances are in the range 0.002–20 mg/kg food, and the maximum use levels are in the range 0.02–100 mg/kg (EFFA, 2003, 2004a, 2012b; Flavour Industry, 2009).

The mTAMDI values for the 10 candidate substances from structural class I for which data have been provided (see Section 7) range from 2 to 5 000 µg/person/day.

For detailed information on use levels and intake estimations based on the mTAMDI approach, see Section 7 and Appendix C.

5. Absorption, distribution, metabolism and elimination

All 12 candidate substances in this group evaluation contain a monocyclic, bicyclic or tricyclic alicyclic moiety with substituents containing a primary alcohol, aldehyde, carboxylic acid or ester function. The evaluation of the metabolism and other aspects of kinetics of the candidate substances in this Flavouring Group Evaluation depend entirely on information on structurally related substances (see Table 7 and Appendix D) and on general knowledge on biochemistry and biotransformation of xenobiotic substances.

It is expected that the three esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids. It is also anticipated that these hydrolysis products may be absorbed and that any remaining unhydrolysed flavouring esters, after absorption, will be hydrolysed in the liver. Gastrointestinal absorption can also be expected for the alcohols, carboxylic acids and the aldehydes in the present group.

The metabolic fate of the three component alcohols, the four candidate alcohols and the four aldehydes in this flavouring group is not completely elucidated. It can be expected that oxidation of the hydroxyl group or aldehyde group will result in the formation of carboxylic acids which can be conjugated and excreted. Alternatively, the component or free alcohols in this group may be conjugated to glucuronide or sulphate without any further oxidation. Further, the cyclohexene derivatives may undergo allylic hydroxylation of the ring and then possible oxidation to keto groups or conjugation with glucuronic acid. These polar metabolites are expected to be excreted in the urine. Three substances [FL-nos: 05.198, 02.216 and 02.217] have a double bond in the side-chain. This is not anticipated to alter the major metabolic pathways outlined above.

Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated. Hence, it may be expected that the candidate substances in this flavouring group are absorbed and metabolised to innocuous products, which are excreted.

For more detailed information, see Appendix D.

6. Application of the procedure for the safety evaluation of flavouring substances

The application of the Procedure is based on intakes estimated on the basis of the MSDI approach. Where the mTAMDI approach indicates that the intake of a flavouring substance might exceed its corresponding threshold of concern, a formal safety assessment is not carried out using the Procedure. In these cases the Panel requires more precise data on use and use levels. For comparison of the intake estimations based on the MSDI approach and the mTAMDI approach, see Section 7.

For the safety evaluation of the 12 candidate substances from chemical groups 1 and 7, the Procedure as outlined in Annex I was applied, based on the MSDI approach. The stepwise evaluations of the substances are summarised in Table 5.

Step 1

All 12 candidate substances are assigned to structural class I according to the decision tree approach presented by Cramer et al. (1978).

Step 2

It is anticipated that the three esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids, and that the component alcohols and carboxylic acids as well as the four candidate alcohols, the four aldehydes and the carboxylic acid will be metabolised to innocuous products at the estimated levels of intake and, accordingly, proceed via the A-side of the Procedure.

Step A3

The estimated European daily per capita intakes from use as flavouring substances of the 12 candidate substances, which have all been assigned to structural class I, range from 0.011 to 43 µg. These estimated intakes are below the threshold of concern of 1 800 µg/person/day for structural class I.

Accordingly, the substances would not be expected to be of safety concern at their estimated levels of intake based on the MSDI approach.

7. Comparison of the intake estimations based on the MSDI approach and the mTAMDI approach

The MSDI ranges from 0.011 to 43 µg per capita per day. These figures are below the threshold of concern value for substances belonging to structural class I (1 800 µg/person/day).

The estimated intakes of the 10 candidate substance in structural class I for which data have been provided, based on the mTAMDI, range from 2 to 5 000 µg/person/day. For four of the substances [FL-nos: 05.157, 05.182, 05.183 and 05.198], the mTAMDI is below the threshold of concern of 1 800 µg/person/day. For six candidate substances [FL-nos: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829], the mTAMDI exceeds the threshold of concern, and for two substances [FL-nos: 02.216 and 02.217] no information on use levels has been provided.

Thus, for eight substances [FL-nos: 02.134, 02.186, 02.216, 02.217, 08.135, 09.342, 09.670 and 09.829], further information is required. This would include more reliable intake data and then, if required, additional toxicological data.

For comparison of the intake estimates based on the MSDI approach and the mTAMDI approach, see Table 4.

Table 4: Estimated intakes based on the MSDI approach and the mTAMDI approach

FL-no	EU Register name	MSDI (µg/person/day)	mTAMDI (µg/person/day)	Structural class	Threshold of concern (µg/person/day)
02.134	2-Cyclohexylethan-1-ol	0.011	3 900	Class I	1 800
02.186	Myrtanol	0.37	3 900	Class I	1 800
02.216	12-beta-Santalen-14-ol	0.085		Class I	1 800
02.217	12-alpha-Santalen-14-ol	0.11		Class I	1 800
05.157	Isocyclocitral	0.011	1 600	Class I	1 800
05.182	2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde	0.061	2.1	Class I	1 800
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal	0.012	1 600	Class I	1 800
05.198	alpha-Methyl ional	0.011	1 600	Class I	1 800
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid	43	5 000	Class I	1800
09.342	Cyclogeranyl acetate	0.24	3 900	Class I	1 800
09.670	Myrtanyl acetate	0.58	3 900	Class I	1 800
09.829	Ethyl cyclohexyl acetate	0.61	3 900	Class I	1 800

8. Considerations of combined intakes from use as flavouring substances

Because of structural similarities of candidate and supporting substances, it can be anticipated that many of the flavourings are metabolised through the same metabolic pathways and that the metabolites may affect the same target organs. Further, in the event of combined exposure to structurally related flavourings, the pathways could be overloaded. Therefore, combined intake should be considered. As flavourings not included in this FGE may also be metabolised through the same pathways, the combined intake estimates presented here are only preliminary. Currently, the combined intake estimates are based only on MSDI exposure estimates, although it is recognised that this may lead to underestimation of exposure. After completion of all FGEs, this issue should be readdressed.

The total estimated combined daily per capita intake of structurally related flavourings is estimated by summing the MSDI for individual substances.

On the basis of the reported annual production volumes in Europe (EFFA, 2003, 2004a, 2007; EFFA, 2011; Flavour Industry, 2009), the combined estimated daily per capita intake as flavourings of the 12 candidate flavouring substances assigned to structural class I is 45 µg, which does not exceed the threshold of concern for a compound belonging to structural class I of 1 800 µg/person/day.

The 12 candidate substances are structurally related to 18 supporting substances evaluated by the JEFCA at its 59th meeting (JECFA, 2003a). The estimated combined intake (in Europe) is approximately 43 µg/person/day for the 18 supporting substances assigned to structural class I. The total estimated combined intake of candidate and supporting substances (in Europe) would be approximately 88 µg, which does not exceed the threshold of concern for structural class I (1 800 µg/person/day).

9. Toxicity

9.1. Acute toxicity

Studies were available for three of the 12 candidate substances and for nine supporting substances. The oral median lethal dose (LD_{50}) in rats ranges from 1 to 5 270 mg/kg body weight (bw).

The acute toxicity data are summarised in Appendix B (Table B1).

9.2. Subacute, subchronic, chronic and carcinogenicity studies

No studies were available for any of the 12 candidate substances.

One study was available for the supporting substance 2,2,3-trimethylcyclopent-3-en-1-yl acetaldehyde [FL-no: 05.119]. This was a single dose level, 90-day gavage study in rats. The oral dose of 12 mg/kg bw/day did not induce adverse effects in this study.

No carcinogenicity studies could be found either for the 12 candidate substances or for any of the 18 supporting substances.

Repeated-dose toxicity data are summarised in Appendix B (Table B2).

9.3. Developmental/reproductive toxicity studies

No studies on developmental or reproductive toxicity are available either for the 12 candidate substances or for the 18 supporting substances.

9.4. Genotoxicity studies

Data are available for the supporting substance 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931], but no studies on genotoxicity are available for the 12 candidate substances.

2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] did not induce any biologically significant increases in bacterial mutation when evaluated in an Ames test in the presence and absence of S9 metabolic activation. It did induce weak genotoxic effects in the *in vitro* micronucleus assay in an initial experiment in the presence of S9-mix at the highest concentration only. In a second experiment, although statistically significant increases were observed at the lowest and highest concentrations tested, these increases fell within the historical control range for the testing laboratory, and were not considered to be biologically important. The Panel therefore concluded that 2,6-dimethyl-2,5,7-octatriene-1-ol acetate [FL-no: 09.931] does not give rise to concern with respect to genotoxicity.

As 2,6-dimethyl-2,5,7-octatriene-1-ol acetate is considered representative of the two precursors for α,β-unsaturated alicyclic aldehydes [FL-nos: 02.216 and 02.217] from Subgroup 2.1 of FGE.19 (FGE.207), the genotoxicity concern can also be ruled out for these two substances and, accordingly, they can also be evaluated through the Procedure.

The genotoxic potential of the remaining flavouring substances cannot be fully assessed as the data are limited. However, this does not preclude evaluation of the candidate substances in the present group using the Procedure (SCF, 1999).

The genotoxicity data are summarised in Appendix B (Table B3).

CONCLUSIONS

The present revision of FGE.12, FGE.12Rev4, includes the assessment of two additional candidate substances, 12-beta-santalen-14-ol [FL-no: 02.216] and 12-alpha-santalen-14-ol [FL-no: 02.217], compared with FGE.12Rev3.

Ten of the 12 flavouring substances possess one or more chiral centres and additionally, owing to the presence of a double bond, three of these substances can exist as geometric isomers. For all 10 substances, the stereoisomeric composition has been specified sufficiently.

All of the 12 candidate substances belong to structural class I according to the decision tree approach presented by Cramer et al. (1978).

Four of the flavouring substances in the present group have been reported to occur naturally in essential oils and in a few foods.

According to the default MSDI approach, estimated European daily per capita intakes of the 12 candidate substances in this group resulting from their use as flavouring substances range from 0.011 µg to 43 µg. These estimated intakes are below the threshold of concern of 1 800 µg/person/day for structural class I substances.

On the basis of the reported annual production in Europe (MSDI approach), the combined intake of the 12 candidate substances belonging to structural class I would result in a total intake of 45 µg/person/day. This value is below the threshold of concern for structural class I substances. The total combined intakes of the 18 supporting substances and the 12 candidate substances is approximately 86 µg/person/day, which is below the threshold of concern for structural class I (1 800 µg/person/day).

The genotoxic potential of this group of flavouring substances cannot be fully assessed as the data are limited. However, this does not preclude evaluation of the flavouring substances in the present group using the Procedure.

The 12 candidate substances are expected to be absorbed and metabolised to innocuous products, which will subsequently be excreted. The esters are expected to be hydrolysed to component alcohols and carboxylic acids, and the acids subsequently either oxidised completely or conjugated and excreted. The component alcohols, the candidate alcohols and the candidate aldehydes are expected to be oxidised to carboxylic acids, conjugated and excreted. The candidate substances, which are cyclohexene derivatives, may also undergo allylic ring hydroxylation and possible further oxidation or conjugation before excretion. Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated.

No valid toxicity studies have been provided for any of the candidate substances and only one adequate subchronic study was available on a supporting substance.

It is considered that, on the basis of the default MSDI approach, the 12 candidate substances would not give rise to safety concerns at the estimated levels of intake arising from their use as flavouring substances.

When the estimated intakes were based on the mTAMDI they ranged from 2 to 5 000 µg/person/day for the 10 flavouring substances from structural class I for which use levels have been provided. The intakes were above the threshold of concern for structural class I, of 1 800 µg/person/day, for six flavouring substances [FL-nos: 02.134, 02.186, 08.135, 09.342, 09.670 and 09.829] and below the threshold for four flavouring substances [FL-nos: 05.157, 05.182, 05.183 and 05.198]. For two substances [FL-nos: 02.216 and 02.217] no use levels were submitted. Thus, for six of the 12 flavouring substances considered in this Opinion, the intakes, estimated on the basis of the mTAMDI, exceed the relevant threshold for the structural class to which the flavouring substance has been assigned, and for two substances no use levels were submitted. Therefore, for eight substances more reliable exposure data are required. On the basis of such additional data, these flavouring substances should be reconsidered along the steps of the Procedure. Following this Procedure, additional toxicological data might become necessary. The four substances for which intake estimates according

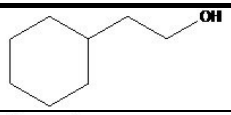
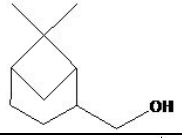
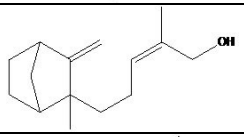
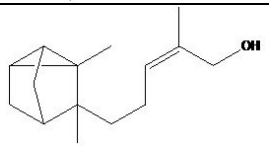
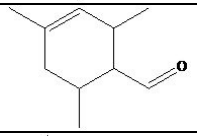
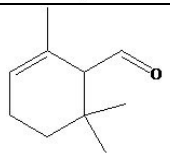
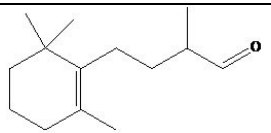
to the mTAMDI approach are below the threshold of concern for structural class I substances are also expected to be metabolised to innocuous products.

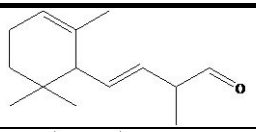
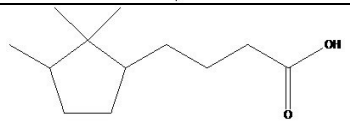
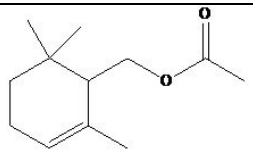
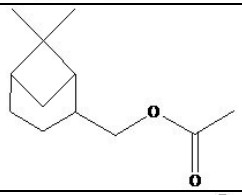
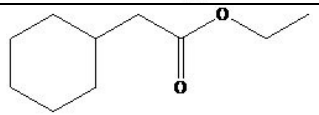
In order to determine whether the conclusion for the 12 candidate substances can be applied to the material of commerce, it is necessary to consider the available specifications. Specifications including complete purity criteria and identity for the materials of commerce have been provided for each of the 12 flavouring substances.

Thus, the Panel concluded that all 12 flavouring substances [FL-nos: 02.134, 02.186, 02.216, 02.217, 05.157, 05.182, 05.183, 05.198, 08.135, 09.342, 09.670 and 09.829] would present no safety concern at the estimated levels of intake based on the MSDI approach.

SUMMARY OF SAFETY EVALUATION

Table 5: Summary of safety evaluation applying the Procedure (based on intakes calculated by the MSDI approach)

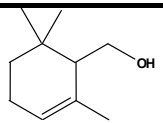
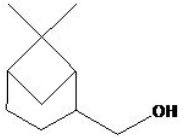
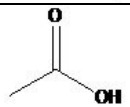
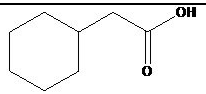
FL-no	EU Register name	Structural formula	MSDI [(a)] (μg/person/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome regarding the named compound [(d) or (e)]	Outcome on the material of commerce [(f), (g) or (h)]	Evaluation remarks
02.134	2-Cyclohexylethan-1-ol		0.011	Class I A3: Intake below threshold	d	f	
02.186	Myrtanol		0.37	Class I A3: Intake below threshold	d	f	
02.216	12-beta-Santalen-14-ol		0.085	Class I A3: Intake below threshold	d	f	
02.217	12-alpha-Santalen-14-ol		0.11	Class I A3: Intake below threshold	d	f	
05.157	Isocyclocitral		0.011	Class I A3: Intake below threshold	d	f	
05.182	2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde		0.061	Class I A3: Intake below threshold	d	f	
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal		0.012	Class I A3: Intake below threshold	d	f	

FL-no	EU Register name	Structural formula	MSDI [(a)] (µg/person/day)	Class [(b)] Evaluation procedure path [(c)]	Outcome regarding the named compound [[(d)] or [(e)]]	Outcome on the material of commerce [[(f)], [(g)] or [(h)]]	Evaluation remarks
05.198	alpha-Methyl ional		0.011	Class I A3: Intake below threshold	d		
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid		43	Class I A3: Intake below threshold	d	f	
09.342	Cyclogeranyl acetate		0.24	Class I A3: Intake below threshold	d	f	
09.670	Myrtanyl acetate		0.58	Class I A3: Intake below threshold	d	f	
09.829	Ethyl cyclohexyl acetate		0.61	Class I A3: Intake below threshold	d	f	

(a): EU MSDI: amount added to food as flavour in (kg/year) × 10^9/(0.1 × population in Europe (= 375 × 10^6) × 0.6 × 365) = µg/person/day.
(b): Thresholds of concern: class I = 1 800 µg/person/day, class II = 540 µg/person/day, class III = 90 µg/person/day.
(c): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.
(d): Compound considered of no safety concern based on intake calculated by the MSDI approach.
(e): Data must be available on the substance or closely related substances to perform a safety evaluation.
(f): No safety concern at the estimated level of intake of the material of commerce meeting the specification requirement (based on intake calculated by the MSDI approach).
(g): Tentatively regarded as presenting no safety concern (based on intake calculated by the MSDI approach) pending further information on the purity of the material of commerce and/or information on stereoisomerism.
(h): No conclusion can be drawn owing to lack of information on the purity of the material of commerce.

EVALUATION STATUS OF HYDROLYSIS PRODUCTS OF CANDIDATE ESTERS

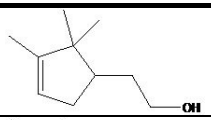
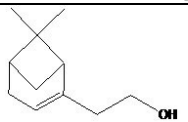
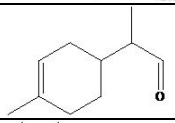
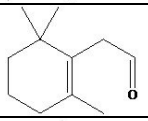
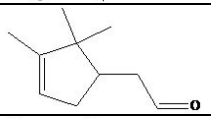
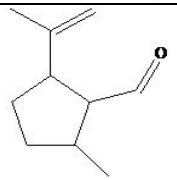
Table 6: Evaluation status of hydrolysis products of candidate esters

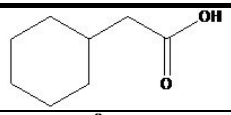
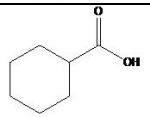
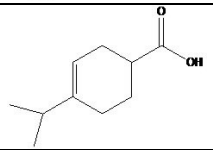
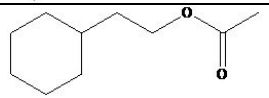
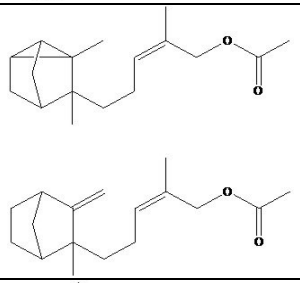
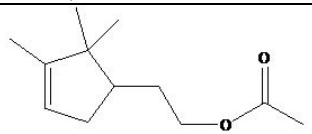
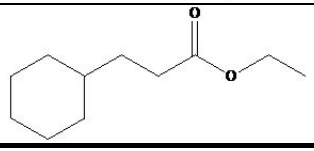
FL-no	EU Register name JECFA no	Structural formula	SCF status [(a)] JECFA status [(b)] CoE status [(c)] EFSA status	Structural class [(d)] Procedure path (JECFA) [(e)]	Comments
	Cyclogeraniol		Not evaluated as flavouring substance		**Not in EU Register**
02.078	Ethanol 41		Category 1 (SCF, 1995)	No evaluation	At the 46th JECFA meeting (JECFA, 1997), the Committee concluded that ethanol posed no safety concern at its current level of intake when ethyl esters are used as flavouring agents
02.186	Myrtanol		FGE.12	Class I A3: Intake below threshold	
08.002	Acetic acid 81		Category 1 (SCF, 1995) Category A (CoE, 1992)	Class I A3, intake above threshold; A4, endogenous	
08.034	Cyclohexylacetic acid 965		Category B (CoE, 1992)	Class I A3: Intake below threshold	

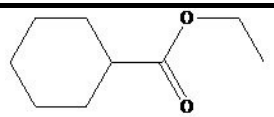
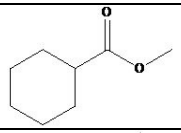
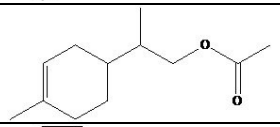
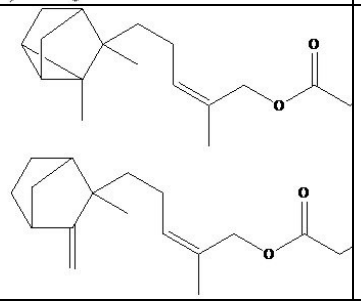
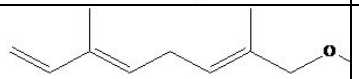
(a): Category 1, considered safe in use; category 2, temporarily considered safe in use; category 3, insufficient data to provide assurance of safety in use; category 4, not acceptable owing to evidence of toxicity.
(b): No safety concern at estimated levels of intake.
(c): Category A, flavouring substance, which may be used in foodstuffs; category B, flavouring substance which can be used provisionally in foodstuffs.
(d): Threshold of concern: class I = 1 800 µg/person/day, class II = 540 µg/person/day, class III = 90 µg/person/day.
(e): Procedure path A substances can be predicted to be metabolised to innocuous products. Procedure path B substances cannot.

SUPPORTING SUBSTANCES SUMMARY

Table 7: Supporting substances summary

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/person/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
02.114	2-(2,2,3-Trimethylcyclopent-3-enyl)ethan-1-ol		3741 1901-38-8	970 JECFA specification (JECFA, 2002b)	0.012		
02.141	2-(6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethan-1-ol		3938 128-50-7	986 JECFA specification (JECFA, 2002b)	33		
05.098	*p*-Menth-1-en-9-al		3178 10347 29548-14-9	971 JECFA specification (JECFA, 2002b)	0.12		
05.112	2,6,6-Trimethylcyclohex-1-en-1-acetaldehyde		3474 10338 472-66-2	978 JECFA specification (JECFA, 2002b)	0.24		
05.119	2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde		3592 10325 4501-58-0	967 JECFA specification (JECFA, 2002b)	5.0		
05.123	5-Isopropenyl-2-methylcyclopentanecarboxaldehyde		3645 55253-28-6	968 JECFA specification (JECFA, 2002b)	0.012		JECFA evaluated cis-5-isopropenyl-cis-methylcyclopentan-1-carboxaldehyde (CAS Registry number as in Register). CAS Registry number in Register refers to the (Z,Z)-isomer

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/person/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
08.034	Cyclohexylacetic acid		2347 34 5292-21-7	965 JECFA specification (JECFA, 2002b)	0.12	Category B (CoE, 1992)	
08.060	Cyclohexanecarboxylic acid		3531 11911 98-89-5	961 JECFA specification (JECFA, 2002b)	0.061		
08.067	1,2,5,6-Tetrahydrocuminic acid		3731 71298-42-5	976 JECFA specification (JECFA, 2002b)	0.012		
09.028	2-Cyclohexylethyl acetate		2348 218 21722-83-8	964 JECFA specification (JECFA, 2002b)	0.97	Deleted (CoE, 1992)	
09.034	Santalyl acetate		3007 224 1323-00-8	985 JECFA specification (JECFA, 2002b)	0.1	Category B (CoE, 1992)	
09.289	alpha-Campholene acetate		3657 36789-59-0	969 JECFA specification (JECFA, 2002b)	0.061		JECFA evaluated campholene acetate (CAS Registry number as in Register)
09.488	Ethyl cyclohexanepropionate		2431 2095 10094-36-7	966 JECFA specification (JECFA, 2002b)	0.12	Deleted (CoE, 1992)	

FL-no	EU Register name	Structural formula	FEMA no CoE no CAS no	JECFA no Specification available	MSDI (EU) [a] (μg/person/day)	SCF status [b] JECFA status [c] CoE status [d]	Comments
09.534	Ethyl cyclohexanecarboxylate		3544 11916 3289-28-9	963 JECFA specification (JECFA, 2002b)	0.24		
09.536	Methyl cyclohexanecarboxylate		3568 11920 4630-82-4	962 JECFA specification (JECFA, 2002b)	0.073		
09.615	*p*-Menth-1-en-9-yl acetate		3566 10748 28839-13-6	972 JECFA specification (JECFA, 2002b)	0.85		
09.712	Santalyl phenylacetate		3008 239 1323-75-7	1022 JECFA specification (JECFA, 2002b)	0.029	Category B (CoE, 1992)	
09.931	2,6-Dimethyl-2,5,7-octatriene-1-ol acetate		3886 999999-91-4	1226 JECFA specification (JECFA, 2003b)	1.2		

(a): EU MSDI: amount added to food as flavouring substance (kg/year) × 10E9/(0.1 × population in Europe (= 375 × 10E6) × 0.6 × 365) = μg/person/day.
(b): Category 1, considered safe in use; category 2, temporarily considered safe in use; category 3, insufficient data to provide assurance of safety in use; category 4, not acceptable owing to evidence of toxicity.
(c): No safety concern at estimated levels of intake.
(d): Category A, flavouring substance which may be used in foodstuffs; category B, flavouring substance which can be used provisionally in foodstuffs.

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Appendix A. Procedure for the safety evaluation

The approach to a safety evaluation of chemically defined flavouring substances as referred to in Commission Regulation (EC) No 1565/2000 (EC, 2000), named the "Procedure", is shown in schematic form in Figure A.1. The Procedure is based on the Opinion of the Scientific Committee on Food expressed on 2 December 1999 (SCF, 1999), which is derived from the evaluation Procedure developed by the Joint FAO/WHO Expert Committee on Food Additives at its 44th, 46th and 49th meetings (JECFA, 1995, 1996, 1997, 1999a).

The Procedure is a stepwise approach that integrates information on intake from current uses, structure–activity relationships, metabolism and, when needed, toxicity. One of the key elements in the Procedure is the subdivision of flavourings into three structural classes (I, II, III) for which thresholds of concern (human exposure thresholds) have been specified. Exposures below these thresholds are not considered to present a safety concern.

Class I contains flavourings that have simple chemical structures and efficient modes of metabolism, which would suggest a low order of oral toxicity. Class II contains flavourings that have structural features that are less innocuous, but are not suggestive of toxicity. Class III comprises flavourings that have structural features that permit no strong initial presumption of safety, or may even suggest significant toxicity (Cramer et al., 1978). The thresholds of concern for these structural classes, 1 800, 540 and 90 µg/person/day, respectively, are derived from a large database containing data on subchronic and chronic animal studies (JECFA, 1996).

In Step 1 of the Procedure, the flavourings are assigned to one of the structural classes. The further steps address the following questions:

- Can the flavourings be predicted to be metabolised to innocuous products[11] (Step 2)?
- Do their exposures exceed the threshold of concern for the structural class (Steps A3 and B3)?
- Are the flavourings or their metabolites endogenous[12] (Step A4)?
- Does a NOAEL exist on the flavourings or on structurally related substances (Steps A5 and B4)?

In addition to the data provided for the flavouring substances to be evaluated (candidate substances), toxicological background information available for compounds structurally related to the candidate substances is considered (supporting substances), in order to ensure that these data are consistent with the results obtained after application of the Procedure.

The Procedure is not to be applied to flavourings with existing unresolved problems of toxicity. Therefore, the right is reserved to use alternative approaches if data on specific flavourings warrant such actions.

[11] "Innocuous metabolic products": products that are known or readily predicted to be harmless to humans at the estimated intakes of the flavouring agent" (JECFA, 1997).

[12] "Endogenous substances": Intermediary metabolites normally present in human tissues and fluids, whether free or conjugated; hormones and other substances with biochemical or physiological regulatory functions are not included (JECFA, 1997).

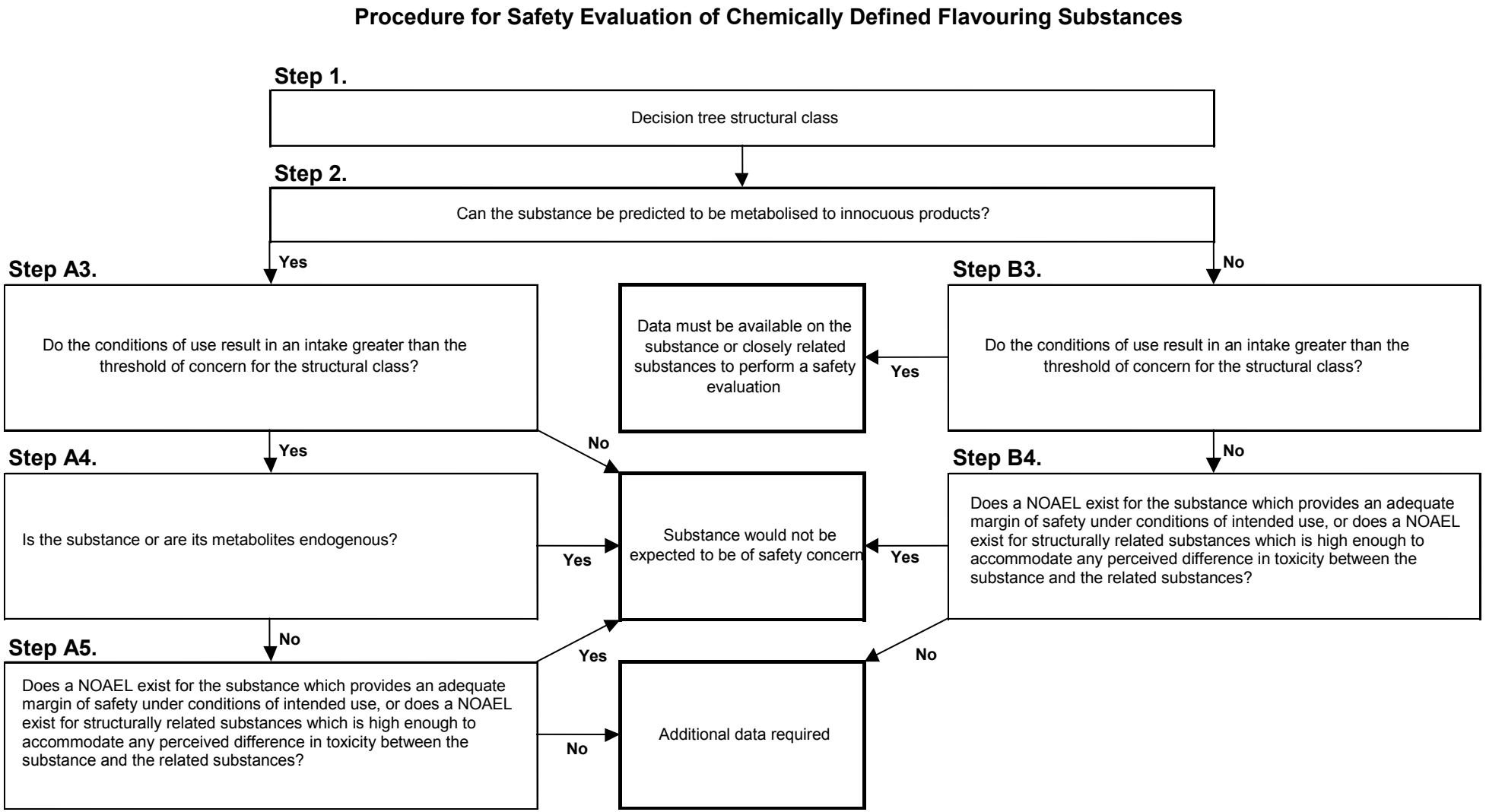


Figure A.1: Procedure for safety evaluation of chemically defined flavouring substances

Appendix B. Toxicity

Oral acute toxicity data are available for three candidate substances of the present Flavouring Group Evaluation, and for nine supporting substances evaluated by the JECFA at the 59th meeting (JECFA, 2003a). The supporting substances are listed in brackets.

Table B1: Acute toxicity

Chemical name [FL-no]	Species	Sex	Route	LD_{50} (mg/kg bw)	Reference	Comments
(Cyclohexanecarboxylic acid [08.060])	Rat	M, F	Gavage	3 265	Moran et al., 1980	Study acceptable but number of dosage groups, and thus number of animals tested, has not been referred.
(Methyl cyclohexanecarboxylate [09.536])	Rat	M, F	Gavage	3 881	Moran et al., 1980	Study acceptable but number of dosage groups has not been referred
(Ethyl cyclohexanecarboxylate [09.534])	Rat	M, F	Gavage	3 962	Moran et al., 1980	Study acceptable but number of dosage groups has not been referred
(Cyclohexaneethyl acetate [09.028])	Rat	NR	Oral	3 200	Wohl, 1974a	Not adequate LD_{50} study
	Rat	NR	Oral	2 190	Moreno, 1978	The study is considered valid
(2,2,3-Trimethylcyclopent-3-en-1-yl acetaldehyde [05.119])	Rat	NR	Oral	4 300	BIBRA, 1976	The LD_{50} value cited is deduced according to Litchfield and Wilcoxon (1949). Another LD_{50} value is also cited in the BIBRA study, 3 900 mg/kg, deduced according to Weill (1952)
	Rat	NR	Oral	4 100	Moreno, 1978	Study acceptable. Substance name is given as 'aldehyde campholenique'
(Campholene acetate [09.289])	Rat	M, F	Gavage	M: 4 640–5 270 F: 3 000	Piccirillo et al., 1979	The study is considered valid
(*alpha*-Campholenic alcohol [02.114])	Rat	NR	Gavage	1 000–2 000	Levenstein, 1982	Study is inadequate for determination of LD_{50}. Also, substance name is only given as code
(1,2,5,6-Tetrahydrocuminic acid [08.067])	Rat	NR	Gavage	> 2 500	Levenstein, 1981	Study inadequate for derivation of LD_{50}. Also, only code name given for substance
4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal [05.183]	Rat	NR	Oral	> 5 000	Moreno, 1977a	Study inadequate for derivation of LD_{50} Also, substance name given as 'cetonal'. It has not been possible to confirm that this is the same substance
(10-Hydroxymethylene-2-pinene [02.141])	Rat	NR	Oral	890	Moreno, 1977b	Study acceptable, but substance name given as Nopol. It has not been possible to confirm that this is the same substance
2-Cyclohexylethan-1-ol [02.134]	Rat	NR	Oral	0.94	Wohl, 1974b	
Isocyclocitral [05.157]	Rat	NR	Oral	4.5 ml/kg bw	Levenstein, 1973	

NR, not reported; M, male; F, female.

Subacute/subchronic/chronic/carcinogenic toxicity data are available for none of the candidate substances of the present Flavouring Group Evaluation but for one supporting substance evaluated by the JECFA at the 59th meeting (JECFA, 2003a). The supporting substance is listed in brackets.

Table B.2. Subacute/subchronic/chronic/carcinogenicity studies

Chemical Name [FL-no]	Species; sex No per group	Route	Dose level	Duration (days)	NOAEL (mg/kg bw/day)	Reference	Comments
(2,2,3-Trimethylcyclopent-3-en-1-yl)acetaldehyde [05.119])	Rat; M, F 8	Gavage	12 mg/kg bw/day	90	12	BIBRA, 1976	Single-dose study

M, male; F, female; NOAEL, no observed effect level.

B.1. Developmental and reproductive toxicity studies

No developmental and reproductive toxicity data are available for the candidate substances of the present Flavouring Group Evaluation or for the supporting substances evaluated by the JECFA at the 59th meeting (JECFA, 2003a) or the 61st meeting (JECFA, 2004b).

B.2. Genotoxicity (*in vitro*)

In vitro mutagenicity/genotoxicity data are available for one structurally related candidate substance. No *in vitro* mutagenicity/genotoxicity data are available for the candidate substances of the present Flavouring Group Evaluation or for the supporting substances evaluated by the JECFA at the 59th meeting (JECFA, 2003a).

Table B.3. Summary of additional genotoxicity data for [FL-no: 09.931] of Subgroup 1.1.2

Chemical name [FL-no]	Test system *in vitro*	Test 0bject	Concentrations of substance and test conditions	Result	Reference	Comments
2,6-Dimethyl-2,5,7-octatriene-1-ol acetate [09.931]	Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA102	5–1 500 µg/plate [(a), (c)] 5–5 000 µg/plate [(b), (c)]	Negative [(a), (c)] Equivocal [(b), (c)]	King, 2000	Reliable without restriction. GLP study in compliance with OECD Guideline 471. A small increase in TA102 revertant numbers was seen at 15 and 50 µg/plate in the presence of S9-mix, but not at higher concentrations
		S. typhimurium TA98, TA100, TA1535, TA1537 and TA102	5–1 500 µg/plate [(a), (c)] 5–5 000 µg/plate [(b), (c)]	Negative [(a), (c)] Negative [(b), (c)]		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the second experiment
		S. typhimurium TA102	5–1 500 µg/plate[(b), (c)]	Negative		The small increase in TA102 revertant numbers seen in the first experiment at 15 and 50 µg/plate in the presence of S9-mix was not reproduced in the third experiment
	Micronucleus assay	Human peripheral blood lymphocytes (male donors)	70–120 µg/mL [(a), (d)] 120–225 µg/mL [(b), (d)] 20–60 µg/mL[(a), (e)] 119.2–290 µg/mL [(b), (d)]	Weak positive +S9 Retest within normal values	Whitwell, 2012	Reliable without restriction. GLP study in compliance with OECD Guideline 487. Weak evidence of inducing micronuclei in the presence of S9-mix in a first experiment (increases only in one culture). A re-test under the same conditions and using a higher top concentration resulted in MNBN frequencies within the historical negative control range at 95th percentile, but were statistically significant due to low vehicle control values

(a): Without S9-mix metabolic activation.
(b): With S9-mix metabolic activation.
(c): Plate incorporation method.
(d): Three-hour incubation with 21-hour recovery period.
(e): Twenty-four-hour incubation with no recovery period.
GLP, good laboratory practice; OECD, Organisation for Economic Co-operation and Development; MNBN, micronucleated binucleate cell.

B.3. Genotoxicity (*in vivo*)

No *in vivo* mutagenicity/genotoxicity data are available for the candidate substances of the present Flavouring Group Evaluation or for the supporting substances evaluated by the JECFA at the 59th meeting (JECFA, 2003a) or the 61st meeting (JECFA, 2004b).

Appendix C. Use levels/mTAMDI

C1. Normal and maximum use levels

For each of the 18 food categories (Table C.1) in which the candidate substances are used, the Flavour Industry reports a "normal use level" and a "maximum use level" (EC, 2000). According to the Industry, "normal use" is defined as the average of reported usages and "maximum use" is defined as the 95th percentile of reported usages (EFFA, 2002). The normal and maximum use levels in different food categories have been extrapolated from figures derived from 12 model flavouring substances (EFFA, 2004b).

Table C.1: Food categories according to Commission Regulation (EC) No 1565/2000 (EC, 2000)

Food category	Description
01.0	Dairy products, excluding products of category 02.0
02.0	Fats and oils, and fat emulsions (type water-in-oil)
03.0	Edible ices, including sherbet and sorbet
04.1	Processed fruit
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds
05.0	Confectionery
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery wares
07.0	Bakery wares
08.0	Meat and meat products, including poultry and game
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms
10.0	Eggs and egg products
11.0	Sweeteners, including honey
12.0	Salts, spices, soups, sauces, salads, protein products, etc.
13.0	Foodstuffs intended for particular nutritional uses
14.1	Non-alcoholic ("soft") beverages, excluding dairy products
14.2	Alcoholic beverages, including alcohol-free and low-alcoholic counterparts
15.0	Ready-to-eat savouries
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)—foods that could not be placed in categories 01.0–15.0

The "normal and maximum use levels" are provided by Industry for 10 candidate substances in the present flavouring group (Table C.2).

Table C.2: Normal and maximum use levels (mg/kg) for the candidate substances in FGE.12Rev4 (EFFA, 2003, 2004a, 2007, 2012b; Flavour Industry, 2009)

FL-no	Food categories																	
	Normal use levels (mg/kg) Maximum use levels (mg/kg)																	
	01.0	**02.0**	**03.0**	**04.1**	**04.2**	**05.0**	**06.0**	**07.0**	**08.0**	**09.0**	**10.0**	**11.0**	**12.0**	**13.0**	**14.1**	**14.2**	**15.0**	**16.0**
02.134	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
02.186	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
05.157	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
05.182	– –	– –	0.01 0,1	0.005 0.05	– –	0.005 0.05	– –	0.006 0.06	– –	– –	– –	– –	– –	– –	0.002 0.02	0 0	– –	– –
05.183	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
05.198	3 15	2 10	3 15	2 10	– –	4 20	2 10	5 25	1 5	1 5	– –	– –	2 10	3 15	2 10	4 20	5 25	2 10
08.135	10 30	– –	– –	– –	– –	10 40	– –	– –	– –	– –	– –	10 40	– –	– –	10 30	10 40	– –	10 40
09.342	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.670	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25
09.829	7 35	5 25	10 50	7 35	– –	10 50	5 25	10 50	2 10	2 10	– –	– –	5 25	10 50	5 25	10 50	20 100	5 25

C2. mTAMDI calculations

The method for calculation of mTAMDI values is based on the approach used by SCF up to 1995 (SCF, 1995). The assumption is that a person may consume the amount of flavourable foods and beverages listed in Table C.3. These consumption estimates are then multiplied by the reported use levels in the different food categories and summed up.

Table C.3: Estimated amount of flavourable foods, beverages, and exceptions assumed to be consumed per person per day (SCF, 1995)

Class of product category	Intake estimate (g/day)
Beverages (non-alcoholic)	324.0
Foods	133.4
Exception a: candy, confectionery	27.0
Exception b: condiments, seasonings	20.0
Exception c: alcoholic beverages	20.0
Exception d: soups, savouries	20.0
Exception e: others, e.g. chewing gum	e.g. 2.0 (chewing gum)

The mTAMDI calculations are based on the normal use levels reported by Industry. The seven food categories used in the SCF TAMDI approach (SCF, 1995) correspond to the 18 food categories as outlined in Commission Regulation (EC) No 1565/2000 (EC, 2000) and reported by the Flavour Industry in the following way (see Table C.4):

- Beverages (SCF, 1995) correspond to food category 14.1 (EC, 2000)
- Foods (SCF, 1995) correspond to the food categories 1, 2, 3, 4.1, 4.2, 6, 7, 8, 9, 10, 13 and/or 16 (EC, 2000)
- Exception a (SCF, 1995) corresponds to food categories 5 and 11 (EC, 2000)
- Exception b (SCF, 1995) corresponds to food category 15 (EC, 2000)
- Exception c (SCF, 1995) corresponds to food category 14.2 (EC, 2000)
- Exception d (SCF, 1995) corresponds to food category 12 (EC, 2000)
- Exception e (SCF, 1995) corresponds to others, e.g. chewing gum.

Table C.4: Distribution of the 18 food categories listed in Commission Regulation (EC) No 1565/2000 (EC, 2000) into the seven SCF food categories used for TAMDI calculation (SCF, 1995)

	Food categories according to Commission Regulation 1565/2000	**Distribution of the seven SCF food categories**		
Key	**Food category**	**Food**	**Beverages**	**Exceptions**
01.0	Dairy products, excluding products of category 02.0	Food		
02.0	Fats and oils, and fat emulsions (type water-in-oil)	Food		
03.0	Edible ices, including sherbet and sorbet	Food		
04.1	Processed fruit	Food		
04.2	Processed vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes), and nuts and seeds	Food		
05.0	Confectionery			Exception a
06.0	Cereals and cereal products, including flours and starches from roots and tubers, pulses and legumes, excluding bakery wares	Food		
07.0	Bakery wares	Food		
08.0	Meat and meat products, including poultry and game	Food		
09.0	Fish and fish products, including molluscs, crustaceans and echinoderms	Food		
10.0	Eggs and egg products	Food		
11.0	Sweeteners, including honey			Exception a
12.0	Salts, spices, soups, sauces, salads, protein products, etc.			Exception d
13.0	Foodstuffs intended for particular nutritional uses	Food		
14.1	Non-alcoholic ("soft") beverages, excluding dairy products		Beverages	
14.2	Alcoholic beverages, incl. alcohol-free and low-alcoholic counterparts			Exception c
15.0	Ready-to-eat savouries			Exception b
16.0	Composite foods (e.g. casseroles, meat pies, mincemeat)— foods that could not be placed in categories 01.0–15.0	Food		

The mTAMDI values (see Table C.5) are presented for 10 flavouring substances in the present flavouring group, for which Industry has provided use and use levels (EFFA, 2003, 2004a, 2007, 2012b; Flavour Industry, 2009). The mTAMDI values are only given for the highest reported normal use levels.

Table C.5: Estimated intakes based on the mTAMDI approach

FL-no	**EU Register name**	**mTAMDI (μg/person/day)**	**Structural class**	**Threshold of concern (μg/person/day)**
02.134	2-Cyclohexylethan-1-ol	3 900	Class I	1 800
02.186	Myrtanol	3 900	Class I	1 800
02.216	12-beta-Santalen-14-ol		Class I	1 800
02.217	12-alpha-Santalen-14-ol		Class I	1 800
05.157	Isocyclocitral	1 600	Class I	1 800
05.182	2,6,6-Trimethylcyclohex-2-ene-1-carboxaldehyde	2.1	Class I	1 800
05.183	4-(2,6,6-Trimethylcyclohexenyl)-2-methylbutanal	1 600	Class I	1 800
05.198	alpha-Methyl ional	1 600	Class I	1 800
08.135	4-(2,2,3-Trimethylcyclopentyl)butanoic acid	5 000	Class I	1 800
09.342	Cyclogeranyl acetate	3 900	Class I	1 800
09.670	Myrtanyl acetate	3 900	Class I	1 800
09.829	Ethyl cyclohexyl acetate	3 900	Class I	1 800

Appendix D. Metabolism

D1. Introduction

The 12 candidate flavouring substances in this group evaluation are 2-cyclohexylethan-1-ol [FL-no: 02.134], 12-beta-santalen-14-ol [FL-no: 02.216], 12-alpha-santalen-14-ol [FL-no: 02.217] myrtanol and its acetate [FL-nos: 02.186 and 09.670, respectively], four aldehydes, isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] and alpha-methyl ional [FL-no: 05.198], one acid, 4-(2,2,3-trimethylcyclopentyl)butanoic acid [FL-no: 08.135], and ethyl cyclohexyl acetate [FL-no: 09.829] as well as the acetate of cyclogeraniol [FL-no: 09.342]. For none of these candidate substances, were absorption, distribution, metabolism or elimination studies available. The assessment of the toxicokinetic properties of this group of substances relies therefore on general knowledge about biotransformation and data for representatives of a group of 18 structurally related (supporting) substances, 16 of which were evaluated during the 59th JECFA meeting as "Alicyclic primary alcohols, aldehydes, acids, and related esters", one of which was evaluated, also at the 59th JECFA meeting, as a member of the group "Phenethyl alcohol, aldehyde, acid and related acetals and esters" (JECFA, 2002a, 2003a) and one of which [FL-no: 09.931] was evaluated at the 61st JECFA meeting as being in the group "Aliphatic, branched-chain saturated and unsaturated alcohols, aldehydes, acids, and related esters" (JECFA, 2004a, b).

D2. Absorption, distribution, metabolism and elimination

D2.1. Ester hydrolysis

Two of the candidate substances in this Flavouring Group Evaluation are esters of alicyclic alcohols and acetic acid, cyclogeranyl acetate [FL-no: 09.342] and myrtanyl acetate [FL-no: 09.670], and one is an ester of alicyclic carboxylic acid and ethanol, ethyl cyclohexyl acetate [FL-no: 09.829], which can be expected to be subject to hydrolysis.

Ester hydrolysis is catalysed by classes of enzymes known as carboxylesterases (Graffner-Nordberg et al., 1998), the most important of which are the B-esterases. Although these enzymes are present in most mammalian tissues, they predominate in the liver (Heymann, 1980; Graffner-Nordberg et al., 1998). The substrate specificity of B-carboxylesterase isoenzymes has been correlated with the structure of the alcohol and acid components (Heymann, 1980). The aliphatic esters hydrolyse rapidly in liver homogenate, simulated pancreatic fluid, simulated gastric fluid and preparations of intestinal mucosa *in vitro* (Leegwater and van Straten, 1974a, b; Longland et al., 1977; Junge and Heymann, 1979; Grundschober, 1977; Graffner-Nordberg et al., 1998). The results of *in vitro* studies indicate that the affinity of the esterases for their substrates increases as the length of the ester increases and that the rate of hydrolysis of the straight-chain esters is approximately 100 times higher than the rate of hydrolysis of the branched-chain esters (Arndt and Krisch, 1973; Butterworth et al., 1975; Junge and Heymann, 1979).

Cyclohexanecarboxylate methyl ester and cyclohexanecarboxylate ethyl ester were incubated separately with 50 mL of simulated gastric fluid at 37 °C for six hours. The results showed approximately 20 % hydrolysis of each ester in the gastric fluid system. After a five-hour incubation in simulated intestinal fluid, approximately 40 % of cyclohexanecarboxylate methyl ester and 50 % cyclohexanecarboxylate ethyl ester were hydrolysed (Moran and Tyburcy, 1979). In an *in vitro* hydrolysis study, 100 % of cyclohexanepropionate ethyl ester was hydrolysed after two hours' incubation in 5 % pancreatin (Leegwater and van Straten, 1974a; Grundschober, 1977).

The *in vitro* hydrolysis of the structurally related ester *p*-1-(7)8-menthadien-2-yl acetate[13] was investigated in rat liver homogenate. After incubation of this substance in homogenate at 37 °C for 15, 30 and 60 minutes, complete (100 %) hydrolysis was observed after 60 minutes, with 92 % hydrolysis occurring within the first 15 minutes (Salzer, 1998).

These data indicate that after oral exposure, the three candidate esters in this group of flavouring substances [FL-nos: 09.342, 09.670 and 09.829] will be hydrolysed either prior to absorption by enzymes in the gastrointestinal tract or after absorption by esterases in the liver to yield their component alcohols and carboxylic acids. The component acid (acetic acid) from two of these esters [FL-nos: 09.342 and 09.670] has been evaluated previously (e.g. FGE.01 or FGE.02) and the component ethanol from [FL-no: 09.829] (the JECFA had concluded that ethanol poses no safety concern at its current level of intake when ethyl esters are used as flavouring agents (JECFA, 1997)) will not be further discussed in this FGE.

D2.2. Absorption, distribution and excretion

For the candidate substances, data on absorption, distribution and excretion are not available. Some data are available on the sodium salt of the supporting substance cyclohexanecarboxylic acid [FL-no: 08.060][13] and on the related substance perillyl alcohol.[13]

D2.2.1. Cyclohexanecarboxylic acid[13]

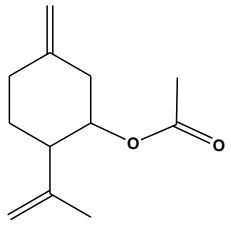
Cyclohexanecarboxylate sodium salt containing a ^{14}C-labelled ring was orally administered to male Wistar albino rats at a dose of 100 mg/kg bw. The results showed that > 98 % of the original dose was excreted as urinary metabolites within seven hours. Less than 1 % was excreted via the faeces or expired air (Brewster et al., 1977).

Cyclohexanecarboxylic acid and 1-methyl-1-cyclohexanecarboxylate were studied in bile duct- and urinary tract-cannulated rats. Female Sprague–Dawley rats (four rats per compound) were administered via intravenous infusion a 0.52 mmol/kg bw bolus dose of cyclohexanecarboxylic acid (66 mg/kg bw) or 1-methyl-1-cyclohexanecarboxylate (73 mg/kg bw), followed by a 0.3-mL saline flush for each rat. Hardly any parent substance was excreted into urine or bile. Biliary excretion of base-labile (presumably glucuronide) conjugates accounted for approximately 5 and 59 %, and urinary excretion accounted for 12 and 25 % of the systemic elimination of cyclohexanecarboxylate and 1-methyl-1-cyclohexanecarboxylate, respectively. The authors concluded that enterohepatic circulation of 1-methyl-1-cyclohexanecarboxylic acid, but not of cyclohexanecarboxylic acid itself, occurs (Liu et al., 1992).

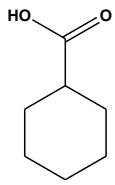
D2.2.2. Perillyl alcohol[13]

The kinetics of *p*-mentha-1,8-dien-7-ol (i.e. perillyl alcohol) has been studied in rats, dogs and humans. This substance is most closely related to *p*-mentha-1,8(10)-dien-9-ol and its acetate [FL-nos: 02.122 and 09.809, respectively] (Subgroup 2.1 of FGE.19 (EFSA, 2008)).

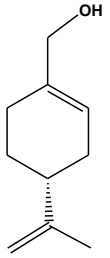
Within four hours after a single dose of 1 000 mg perillyl alcohol/kg bw, administered to female Wistar–Furth rats via gavage, major plasma metabolites were identified as oxidised metabolites of



13 p-1-(7)8-menthadien-2-yl acetate



cyclohexanecarboxylic acid



perillyl alcohol

perillyl alcohol (perillic acid and dihydroperillic acid). No trace of perillyl alcohol was detected in the plasma at any time point, including 15 minutes post gavage (Haag and Gould, 1994).

Two beagle dogs (male and female) administered 250 mg perillyl alcohol/kg bw by gavage exhibited peak plasma levels of oxidised metabolites of perillyl alcohol (i.e. perillic acid and dihydroperillic acid) at one and five hours post administration, respectively. Analysis of blood specimens collected before dosing and at 19 time points ranging from 10 minutes to 48 hours after dosing indicated the presence of the oxidised metabolites 10 minutes post administration. The parent substance, perillyl alcohol, was undetectable in the plasma (Phillips et al., 1995).

Patients with various advanced malignancies were treated orally with doses of 800, 1 600 or 2 400 mg perillyl alcohol/m^2/dose (equivalent to *c.* 32, 64 or 96 mg/kg bw/dose, assuming a body mass index of 25 kg/m^2). On the first day only a single dose was given, but thereafter the treatment was continued for four weeks, but on a three doses per day basis. Kinetic parameters were determined after the first and last dose. The parent drug was not detected in the plasma. Peak plasma levels (C_{max}) of the two main metabolites of perillyl alcohol occurred at 1.5–3.5 hours (perillic acid) and 3–5 hours (dihydroperillic acid) post administration. Plasma elimination half-lives of the two metabolites studied were 1–6 hours and 2–3 hours, respectively. Repeated dosing did not affect C_{max} values or the area under the curve (AUCs) values for these two metabolites, but there was a clear "levelling of" of C_{max} and AUC values when the dose of the metabolites increased from 1 600 to 2 400 mg/m^2. Urinary metabolites were collected from the patients treated with the 2 400 mg/m^2/dose up to 24 hours after the first dose or up to six hours after the last dose. In both cases ~ 1 % of the dose was collected as unchanged perillic alcohol. Approximately 10 % of the dose was recovered, less than 10 % of which was unchanged parent substance (Ripple et al., 1998).

As part of a phase I dose-escalation trial, perillyl alcohol was administered per os (p.o.) at 800, 1 200 or 1 600 mg/m^2/dose (equivalent to *c.* 32, 48, or 64 mg/kg bw/dose, assuming a body mass index of 25 kg/m^2) to 16 patients with advanced refractory malignancies on a four times per day continuous basis for four weeks to characterise its kinetic profile, maximum tolerated dose, toxicity and antitumour activity. There appeared to be a dose-dependent increase in the plasma levels of the two main metabolites, perillic acid and dihydroperillic acid (see below). There was a trend towards decreasing metabolite levels on day 29 compared with days 1 and 2. Peak metabolite levels were seen one to three hours post administration and metabolite half-lives were about two hours. No indication of dose-related effects on the kinetics was obtained. Approximately 9 % of the total dose was recovered in the urine in the first 24 hours. Only ~ 0.1 % of the dose was recovered as parent substance (Ripple et al., 2000).

From the above-mentioned studies it can be concluded that in humans, dogs and rats orally administered perillyl alcohol is rapidly absorbed and metabolised after ingestion.

D2.3. Biotransformation

D2.3.1. Cyclohexyl derivatives

Metabolism studies conducted on representative flavouring agents indicate that these substances are metabolised primarily by oxidation of the primary alcohol or aldehyde function to yield the corresponding carboxylic acid or oxidation of the alkyl ring substituents to yield polyoxygenated polar metabolites that are readily excreted.

The metabolic options available to alicyclic substances increase as the number and types of functional groups and ring substituents in the molecule increase. If a primary alcohol, aldehyde or carboxylic acid function is present on an alkyl side-chain of the ring, the substance may undergo beta-oxidation at the side-chain. For the present group of flavouring substances, this seems in particular important for [FL-no: 05.183 and 08.135], because these are the only ones with a side-chain which might be shortened by beta-oxidation. If the number of carbons present in the side-chain is odd, beta-oxidative

cleavage cannot continue beyond the point of side-chain attachment but the resulting carboxylic acids may be conjugated with glucuronic acid or glycine (Bernhard and Caflisch-Weill, 1945; Williams, 1959; Brewster et al., 1977).

D2.3.2. Terpenoid primary alcohols and aldehydes

An indication of the metabolic fate of the monocyclic and bicyclic terpenoid aldehydes and alcohols (e.g. candidate substances myrtanol [FL-no: 02.186], isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], cyclogeranyl acetate [FL-no: 09.342] and myrtanyl acetate [FL-no: 09.670] and supporting substances) can be obtained from the biotransformations of representative supporting substance aldehydes, *p*-mentha-1,8-dien-7-al (i.e. perillaldehyde) and 2-formyl-6,6-dimethylbicyclo[3.1.1]hept-2-ene (i.e. myrtenal), which are described below. In addition, for the metabolism of the flavouring substances isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183], alpha-methyl ional [FL-no: 05.198] and cyclogeranyl acetate [FL-no: 09.342], in which multiple and cycloalkene methyl side-chains occur, the metabolism of isophorone (3,5,5-trimethylcylohex-2-ene-1-one [FL-no: 07.126]),[14] alpha-ionone [FL-no: 07.007][14] and beta-ionone [FL-no: 07.008][14] might be used as an example.

D2.3.3. Isophorone

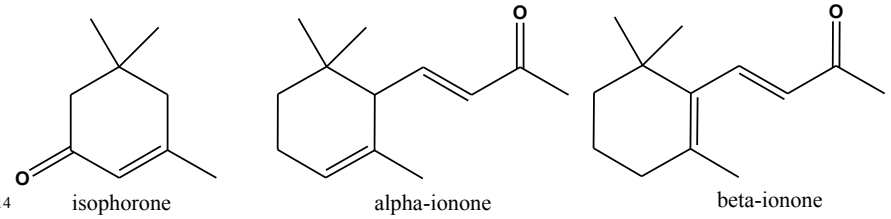
When isophorone[11] was given to rabbits at an oral dose of 1 g/kg bw, glucuronic acid conjugates could be detected in the urine, and, after treatment of the urine with hydrochloric acid, the metabolite 5,5-dimethyl-cyclohex-1-ene-3-one-1-carboxylic acid was found. This shows that for these substances oxidation of the methyl side-chain is a possible metabolic pathway, which, probably via alcohol and aldehyde intermediates, leads to formation of free or conjugated carboxylic acid end products (Truhaut et al., 1970).

D2.3.4. Alpha- and beta-ionone[14]

The candidate substances 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182], alpha-methyl ional [FL-no: 05.198] and cyclogeranyl acetate [FL-no: 09.342] are structurally related to alpha-ionone [FL-no: 07.007],[11] and 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183] is structurally related to beta-ionone [FL-no: 07.008].[11] Available metabolic data on these two ionones indicate that they may undergo allylic ring hydroxylation and possible further oxidation to keto groups. These reactions result in the formation of polar metabolites, which are excreted in the urine unchanged or conjugated with glucuronic acid (JECFA, 1999b). It is anticipated that the four candidate substances [FL-nos: 05.182, 05.183, 05.198 and 09.342] may, at least to some extent, form similar polar metabolites and be excreted in the urine.

D2.3.5. Perillyl alcohol and perillaldehyde

The metabolism of perillyl alcohol, perillaldehyde and perillic acid was determined after intravenous injection in male Wistar rats and in exposed isolated rat hepatocytes. Although perillaldehyde can react spontaneously with glutathione (GSH), no indication of the formation of GSH conjugates was found either *in vivo* or in hepatocytes. After dosing with perillaldehyde, about 50 % of the doses were recovered as glucuronides in bile and urine. From perillic acid only the acyl glucuronide was generated, whereas perillyl alcohol and perillaldehyde formed both acyl and ether glucuronides. The results, together with those of studies in which alcohol dehydrogenase or aldehyde dehydrogenase was



14 isophorone alpha-ionone beta-ionone

inhibited, indicate that perillaldehyde is a major intermediary metabolite of perillyl alcohol in the rat *in vivo* and in rat hepatocytes *in vitro* (Boon et al., 2000).

Six male rabbits were administered *p*-mentha-1,8-dien-7-al (perillaldehyde) orally at a dose level of 2 000 mg per animal. Urine was collected for three consecutive days, pooled and treated with glucuronidase/aryl sulphatase. The neutral urinary fraction contained two metabolites accounting for 7 % of the total amount of parent substance administered. These metabolites were identified as (-)-perillyl alcohol and (–)-cis-shisool (i.e. *para*-menth-8-en-7-ol), representing 46 and 39 % of the neutral metabolites, respectively. The acidic fraction constituted 39 % of the administered amount of perillaldehyde and the two major metabolites in this fraction were perillic acid, which represented 57 % of the acidic urinary metabolites and *p-i*sopropylbenzoic acid (amount not specified). These results indicate that perillaldehyde was oxidised to *p*-mentha-1,8-dien-7-carboxylic acid (i.e. perillic acid). Aromatisation of the cyclohexene ring and reduction of the isopropenyl double bond converted perillic acid in part to *p*-isopropylbenzoic acid. To a lesser extent, *p*-mentha-1,8-dien-7-al was reduced to perillyl alcohol, which can be selectively hydrogenated to yield *p*-mentha-8-en-7-ol (see Figure D.1) (Ishida et al., 1989). Only a relatively small part of the administered dose was recovered. Other metabolites were not mentioned.

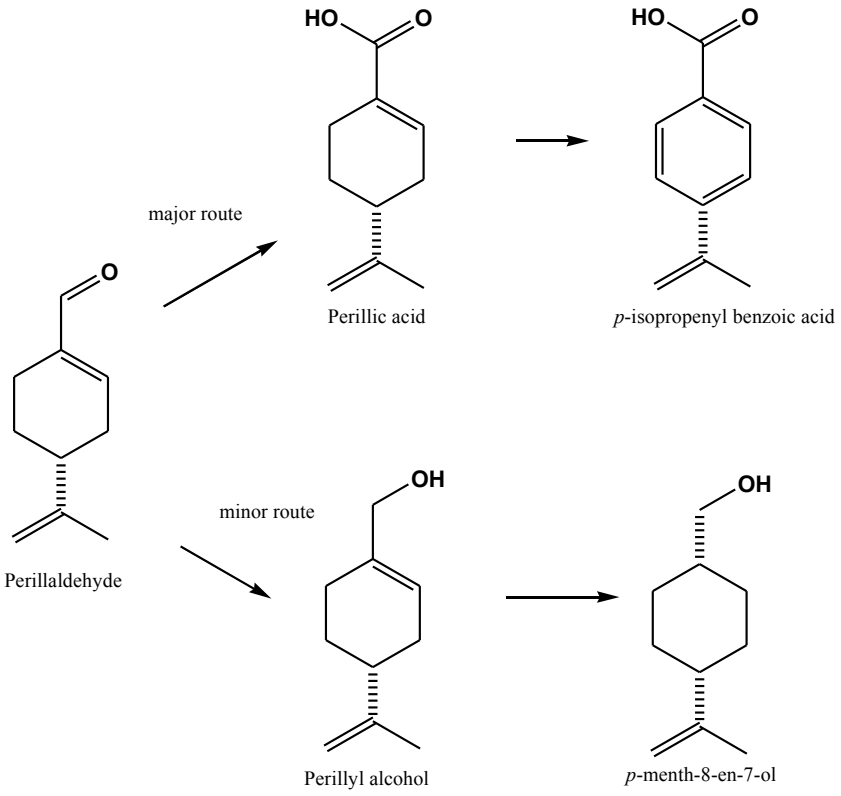


Figure D.1: Metabolism of perillaldehyde in rabbits

Female Wistar–Furth rats were given a single oral dose of 100 mg perillyl alcohol/kg bw by gavage or were given a diet of 2 % perillyl alcohol for a period of 3, 5 or 10 weeks (nominally approximately 1.5 g/kg bw/day). Perillic acid and dihydroperillic acid were detected as major plasma metabolites and perillic acid methyl ester and dihydroperillic acid methyl ester were identified as minor metabolites. The authors concluded that the methyl esters were artefacts formed during processing of urine. Unchanged perillyl alcohol was not detected after the gavage dose, not even at 15 minutes post gavage, nor after subchronic feeding. These results indicate that perillyl alcohol is rapidly absorbed from the gastrointestinal tract and metabolised. The presence of dihydroperillic acid indicates that the

endocyclic double bond was hydrogenated. After acute exposure, the perillic acid/dihydroperillic acid ratio amounted to > 10, while after 3–10 weeks of exposure via the diet this ratio had dropped to 2–3 (Haag and Gould, 1994).

An *in vivo* study conducted in male Wistar rats confirmed that the oxidation of perillyl alcohol to perillic acid involves perillaldehyde as an intermediate. Rats were administered intravenous perillyl alcohol, perillaldehyde or perillic acid at a dose of 80 μmol/kg bw (approximately 12.2, 12.0 or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of perillic acid was the predominant metabolite detected in the urine (10 % of the dose) and bile (46 % of the dose). The glucuronic acid conjugate of perillyl alcohol was also a major biliary metabolite following the intravenous administration of perillyl alcohol (5 %), while urinary excretion of this conjugate amounted to 1 % of the dose. Based on the results, the authors concluded that, within two hours, approximately 56 % of the original dose had been oxidised to perillic acid through perillaldehyde, and was eventually excreted as a glucuronic acid conjugate (Boon et al., 2000).

Patients with various advanced malignancies were treated orally with one dose, followed by three daily doses, of 2 400 mg perillyl alcohol/m^2 (equivalent to *c*. 96 mg/kg bw, assuming a body mass index of 25 kg/m^2) on the following 29 days. Urinary metabolites were collected up to 24 hours after the first dose or up to 6 hours after the last dose. In both cases ~ 1 % of the dose was collected as unchanged perillic alcohol. Two metabolites were found, which constituted approximately 9 % of the dose,of which perillic acid accounted for ~ 90 % and dihydroperillic acid for ~ 10 %. Other metabolites were not monitored (Ripple et al., 1998).

As part of a phase I dose-escalation trial, perillyl alcohol was administered p.o. at 1 200 or 1 600 mg/m^2/dose (equivalent to *c*. 48 or 64 mg/kg bw/dose, assuming a body mass index of 25 kg/m^2) to 16 patients with advanced refractory malignancies on a four times per day continuous basis for four weeks. Approximately 9 % of the total dose was recovered in the urine in the first 24 hours on the first day of treatment and slightly more was recovered on day 15 or day 29 during 6 hours post dosing. At all time points, perillic acid accounted for approximately 80–85 % of recovered metabolites and dihydroperillic acid for 10–17 %. Only about 1 % of the dose was recovered as parent substance. Other metabolites were not monitored (Ripple et al., 2000).

D2.3.6. Myrtenal

Six male rabbits received an oral dose of 2 000 mg of 2-formyl-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene (= (–)-myrtenal) per animal. Myrtenol, dihydromyrtenol, myrtenic acid and perillic acid could be detected in the urine of these animals. Myrtenol and dihydromyrtenol together accounted for 99 % of the neutral metabolite fraction (5 % of the dose). Myrtenic acid represented 76 % of the acid metabolites detected in the urine, but the amount of perillic acid was not specified. The total acidic fraction of urinary metabolites constituted 24 % of the dose. These results indicate that myrtenal can be metabolised to the corresponding carboxylic acid (myrtenic acid). The presence of perillic acid indicates some cleavage of the strained bicyclic ring. To a lesser extent, the aldehyde can either be reduced to myrtenol, which may be conjugated with glucuronic acid and excreted, or may undergo hydrogenation of the double bond to yield dihydromyrtenol (myrtanol; see Figure D.2), which is one of the candidate substances [FL-no: 02.186] and has been shown to be the major neutral metabolite and is excreted unchanged in the urine (Ishida et al., 1989). Urine was collected for three days post dosing. Only a relatively small proportion of the administered dose was recovered. Other metabolites were not mentioned.

Humans exposed to sawmill dust (Eriksson and Levin, 1990) excreted in the urine the glucuronic acid conjugate of myrtenol (2-hydroxymethyl-6,6-dimethyl-bicyclo[3.1.1]hept-2-ene [FL-no: 02.091], the component alcohol in candidate flavouring substances [FL-no: 09.899 and 09.900]) (Subgroup 2.2 of FGE.19) (EFSA, 2008). The myrtenol was not detected in the sawdust (Eriksson and Levin, 1990), but

could have originated from side-chain oxidation of alpha-pinene (= 2,6,6-trimethyl-bicyclo[3.1.1]hept-2-ene [FL-no: 01.004]) (FGE.78Rev1) (EFSA CEF Panel, 2011; Ishida et al., 1981).

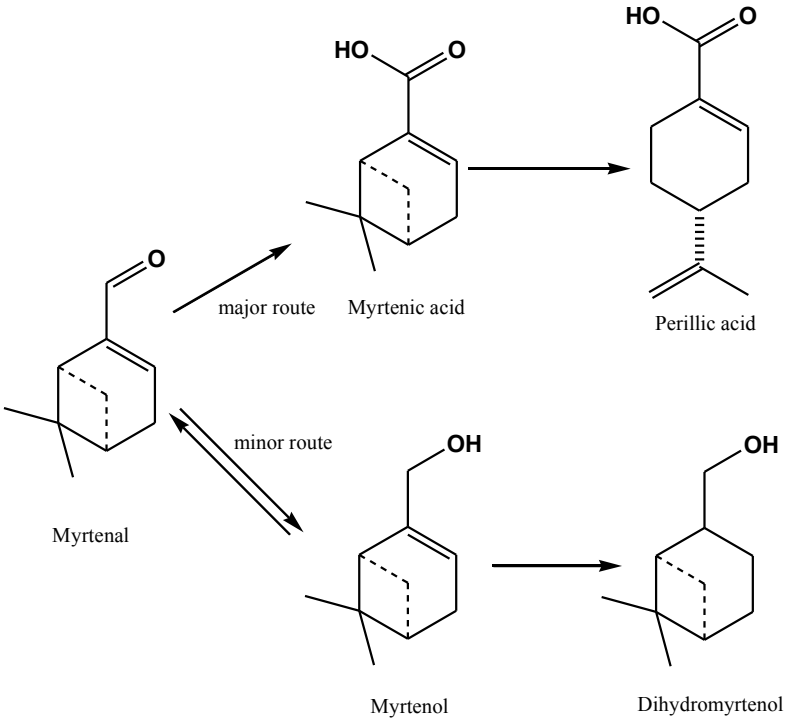


Figure D.2: Metabolism of myrtenal in rabbits

In summary, in mammals, monocyclic or bicyclic terpenoid primary alcohols (e.g. cyclogeraniol [from FL-no: 09.342] and myrtanol [FL-no: 02.186] (and from [FL-no: 09.670], and the structurally related substance perillyl alcohol) are generally oxidised to the corresponding carboxylic acid, conjugated with glucuronic acid and are excreted as urinary metabolites. The same is true for the monocyclic aldehyde (candidate substances isocyclocitral [FL-no: 05.157], 2,6,6-trimethylcyclohex-2-ene-1-carboxaldehyde [FL-no: 05.182] and 4-(2,6,6-trimethylcyclohexenyl)-2-methylbutanal [FL-no: 05.183]) and structurally related substances perillaldehyde and isophorone), which contain alkyl ring substituents. In a minor pathway, the aldehyde may be reduced to the alcohol and excreted as the glucuronide (Ishida et al., 1989; Haag and Gould, 1994). If an endocyclic double bond is present, reduction of this double bond may occur.

D.3. Summary and conclusions

The 12 candidate substances in this group evaluation contain a monocyclic, bicyclic or tricyclic terpenoid moiety, all with a primary oxygenated substituent. The evaluation of the metabolism and other aspects of kinetics of the candidate substances in this Flavouring Group Evaluation depends entirely on information on structurally related substances and on general knowledge of the biochemistry and biotransformation of xenobiotic substances.

It can be expected that the esters in this group will be hydrolysed to yield their component alcohols and carboxylic acids. It can also be expected that these hydrolysis products may be absorbed, and that any remaining unhydrolysed flavouring substance after absorption will be hydrolysed in the liver. Gastrointestinal absorption can also be expected for the free alcohol and the free aldehyde in this group.

The metabolic fate of the component alcohols, the free candidate alcohols and the four aldehydes in this flavouring group is not completely elucidated. It can be expected that oxidation of the hydroxyl group or aldehyde group will result in the formation of carboxylic acids, which can be conjugated and excreted. Alternatively, the component or free alcohols in this group may be conjugated to glucuronide or sulphate without any further oxidation. Further, the cyclohexene derivatives may undergo allylic hydroxylation of the ring and then, perhaps, oxidation to keto groups or conjugation with glucuronic acid. These polar metabolites are expected to be excreted in the urine.

Following absorption, the acids can be expected to be metabolised further via beta-oxidation (if applicable). Alternatively, they can be expected to be conjugated and excreted via the urine.

Neither the chemical structures of the candidate substances in this group nor the metabolic data available suggest that reactive metabolites could be generated. Hence, it may be expected that the candidate substances in this flavouring group are absorbed and metabolised to innocuous products, which are excreted.

ABBREVIATIONS

AUC	area under the curve
bw	body weight
CAS	Chemical Abstract Service
CEF	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
C_{max}	maximum plasma concentration
CoE	Council of Europe
EFFA	European Flavour and Fragrance Association
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FEMA	Flavor and Extract Manufacturers Association
FGE	Flavouring Group Evaluation
FLAVIS (FL)	Flavour Information System (database)
GLP	Good Laboratory Practice
GSH	glutathione
IR	Infrared spectroscopy
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MNBN	micronucleated binucleate cell
MSDI	maximised survey-derived daily intake
mTAMDI	modified theoretical added maximum daily intake
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organization for Economic Co0operation and Development
PCE	Polychromatic erythrocyte
p.o.	per os
SCF	Scientific Committee on Food
WHO	World Health Organization